

PHOSPHOPLASTIN RL

Prothrombin Time Reagent



INTENDED USE

Phosphoplastin RL is intended for use in a one-stage prothrombin time (PT) test on citrated human plasma. The PT test is a quantitative assay used in the general patient population for routine screening to detect deficiencies in the extrinsic pathway of coagulation. The PT test is also used to monitor oral anticoagulant therapy and should be used in a clinical laboratory by qualified laboratory personnel.

SUMMARY AND PRINCIPLE

The one-stage prothrombin time test (PT) has become the basic coagulation screening test for the diagnosis of congenital and acquired deficiencies of Factors II, V, VII and X (1,2).

Oral anticoagulant drugs inhibit hepatic synthesis of the vitamin K dependent clotting Factors II, VII, IX and X. Therefore, the prothrombin time test is appropriate to monitor oral anticoagulant therapy since it is sensitive to three of the four factors involved (3,4). It can be modified to quantitate factor levels using appropriate factor deficient substrate plasmas.

Tissue thromboplastin, in the presence of calcium ions and Factor VII, activates the extrinsic pathway of coagulation. When a mixture of tissue thromboplastin and calcium ions is added to normal anti-coagulated plasma, the clotting mechanism is initiated and a clot will form within a specified time period. If a deficiency exists within the extrinsic pathway, the time required for clot formation will be prolonged. The degree of prolongation is proportional to the severity of single factor deficiency, or in a cumulative deficiency of all the factors involved.

REAGENTS

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY.

1. Phosphoplastin RL Reagent

Ingredients: The reagent contains a liquid saline extract of rabbit brain, calcium ions, preservatives and stabilizers.

Preparation for Use: Reagent comes ready for use. The vial should be inverted several times until a homogeneous suspension is obtained.

Storage and Stability: Reagent should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial. After opening, the original activity is stable for fourteen (14) days at 2 to 8°C.

DO NOT FREEZE

Signs of Deterioration: The reagent is a fine suspension of rabbit brain particles. Large flaky particles in the suspension or prolonged prothrombin times on testing normal plasma or controls may indicate product deterioration.

Instruments

Prothrombin time test may be done by accepted manual methods or by using electro-mechanical or photo-optical coagulation instruments.

Specimen Collection And Handling

NOTE: After initial whole blood collection, during testing all test tubes, syringes and pipettes should be plastic

Specimen: Plasma obtained from whole blood anti-coagulated with 3.2% sodium citrate.

Specimen Collection: Nine parts freshly collected whole blood should be immediately added to one part anticoagulant.

Specimen Preparation: Centrifuge the whole blood specimen at 1500 x g for 15 minutes (NCCLS H21-A4, 2003). Immediately separate the plasma from the red blood cells using a plastic pipette and place it in a plastic test tube at 2 to 8°C until assayed. Perform the prothrombin time test within 2 hours.

Storage and Stability: Before and during testing, the plasma sample should be maintained in the plastic tubes at 2 to 8°C to ensure stability of the factors. If testing is delayed for more than 2 hours, the plasma may be stored at -20°C for two weeks or at -70°C for up to one month. Frozen samples should be thawed rapidly at 37°C before testing.

Materials Provided: Materials needed for prothrombin time tests are provided in the following packaging configurations:

- 10 x 5mL vials Phosphoplastin RL Reagent,
- 10 x 10mL vials Phosphoplastin RL Reagent,

Materials and Equipment Required, but not provided:

1. Coagulation Instrument or 37°C Water Bath and timer
2. Reaction Cups or Plastic test tubes (12 x 75 mm)
3. Pipetting device to deliver 200 µL or 100 µL.
4. Centrifuge
5. Distilled or de-ionized water
6. Control plasmas:
 - PlasmaCon N
 - PlasmaCon L-1
 - PlasmaCon L-2

Test Procedure for PT

NOTE: Throughout the procedure, all test tubes, syringes, and pipettes should be plastic.

I. Automated And Semi-Automated Methods

If using an instrument to perform this test, refer to the appropriate Instrument Operator's Manual for detailed instructions.

II. Manual Method

1. Collect and prepare the blood specimen according to directions outlined in **SPECIMEN COLLECTION AND HANDLING.**
2. Reconstitute the control plasmas according to the package insert included with the control.
3. Perform all tests in duplicate.
4. Prewarm the PT reagent to 37°C for at least 10 minutes.
5. Prewarm 100 µL of the test plasma or control plasma for 2-3 minutes at 37°C.
6. Add 200µL Phosphoplastin RL Reagent to the plasma, simultaneously starting a stopwatch and record the time required for clot formation in seconds.

Quality Control: Each laboratory should establish a quality control program that includes normal and abnormal controls to evaluate instrument, reagent and technologist performance. The normal and abnormal controls should be tested daily prior to performing tests on patient plasmas. Monthly quality control charts (Levy Jennings) are recommended to determine the mean and standard deviation of each of the daily control plasmas.

A normal control such as PlasmaCon N, and abnormal level 1 and level 2 controls such as PlasmaCon L-1 and PlasmaCon L-2 are recommended. If the controls do not perform within their reference ranges, patient results should be considered invalid and not reported.

Results

The results of the Prothrombin Time tests should be reported to the nearest tenth of a second. Results greater than the upper limits of the range should be considered abnormal and follow-up testing should be performed. PT values below the lower limits of the range may indicate a compromised sample, and a new sample should be collected.

Limitations

Expected values for the prothrombin time test will vary from one laboratory to another, depending on several variables. These include the method of clot detection, temperature, pH, sample collection technique, type of anticoagulant and time and method of plasma storage. Therefore, laboratories should establish their own expected values for patients and well defined performance standards for control plasmas. The use of Icteric, Lipemic, or Hemolyzed sample should be avoided due to possible interference especially when using photo-optical instruments. The impact of other therapeutic drugs, in addition to oral anticoagulant therapy, can influence interpretation of PT test results. Obtaining an accurate patient history and noting specific drug therapies can help in the proper understanding of the potential impact on laboratory test results. The presence of heparin as a contaminant in the patient sample must always be considered when an abnormal result is obtained.

EXPECTED VALUES

A reference range study was conducted using triplicate specimens from 120 normal health adults. Approximately equal numbers of males and females were used. The PT results were as follows:

	Mean (seconds)	Range for +/- 2 SD
Photo-optical	12.3	9.7-14.9 seconds
Mechanical	12.6	11.1-14.1 seconds

These values should only serve as guidelines. Because differences may exist between instruments, laboratories, and local populations, it is recommended that each laboratory establish its own reference range of expected prothrombin time results

DETERMINATION OF INR

The World Health Organization (WHO) recommends the use of the International Normalized Ratio (INR) instead of direct reporting of the PT times when monitoring patients undergoing Oral Anticoagulant Therapy. This allows patient results to be compared between different laboratories that may be using reagents with different sensitivities.

The INR is calculated by using the ratio of the Patient PT to the mean of the normal reference range raised to the power of the reagent International Sensitivity Index (ISI).

$$\text{INR} = (\text{Patient PT} / \text{mean of normal range})^{\text{ISI}}$$

The ISI is assigned by comparison to a highly sensitive WHO thromboplastin standard reference material. The lower the ISI for a given reagent the more sensitive the reagent is to coagulation factors. The lot specific ISI value for Phosphoplastin RL can be found on the outer box front panel.

PERFORMANCE CHARACTERISTICS

I. Precision

Precision studies were performed to establish Within-Run and Between-Run CV's for normal control plasma and abnormal control plasma. A single lot number of Phosphoplastin RL reagent was used for these studies. Results are shown below.

		Within Run	Between Run
Photo-optical			
	PlasmaCon N	n 30	15
	Mean	12.7	12.9
	SD	0.1	0.13
	CV	0.7	1.03
PlasmaCon L-2	n	30	15
	Mean	52.9	53.9
	SD	1.0	0.71
	CV	1.9	1.31
Mechanical			
	PlasmaCon N	n 30	15
	Mean	14.4	14.1
	SD	0.3	0.15
	CV	2.1	1.04
PlasmaCon L-2	n	30	15
	Mean	59.7	57.0
	SD	0.9	0.31
	CV	1.5	0.54

II. Comparison

A comparison study was done using the Phosphoplastin RL Reagent and another thromboplastin reagent. One hundred five (105) plasma specimens, both normal and abnormal clinical samples were tested with both PT reagents. The linear regression equation and coefficient of determination (r^2) of the INR values are reported:

Photo-optical

$$N = 105 \quad r^2 = 0.98 \quad Y = 1.065X - 0.1245$$

Mechanical

$$N = 105 \quad r^2 = 0.92 \quad Y = 1.059X - 0.114$$

$$Y = \text{Phosphoplastin RL reagent}$$
$$X = \text{Reference thromboplastin reagent}$$

References:

1. Quick A.J., The Prothrombin Time in Hemophilia and in Obstructive Jaundice. J. Biol. Chem.: 109,73-74; 1935.
2. Biggs R. ed , Human Blood Coagulation Hemostasis and Thrombosis Second Ed. Blackwell Scientific Publications, London 1976.
3. Peterson C.E., Kwaan H.C., Current Concepts of Warfarin Therapy, Arch Intern. Med. 146: 581-584, 1986.
4. Loeliger E.A.: ICEH/ICTH Recommendations for Reporting Prothrombin Time in Oral Anticoagulant Control, Throm. Haemost. 53: 155-156, 1985.

